The University of Chicago

SOME EFFECTS OF CORPUS LUTEUM EXTRACTS AND OF SYNTHETIC PROGESTERONE ON YOUNG MALE ALBINO RATS

A PART OF A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY
1938

By
JULE KITCHELL LAMAR

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SOME EFFECTS OF CORPUS LUTEUM EXTRACTS AND OF SYNTHETIC PROGESTERONE ON YOUNG MALE ALBINO RATS[1,2]

(One plate)

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THE corpus luteum is an endocrine gland which, by means of its internal secretion, affects many processes in the postpuberal female physiology of mammals (Allen, Hisaw, and Gardner, 1939). These processes may be similarly affected in the absence of all luteal tissue by administration of crude potent luteal-tissue extracts or of pure crystalline progesterone, the so-called "hormone of the corpus luteum" (Allen and Corner, 1930; Corner and Allen, 1936). Whereas, all other sex hormones, both male and female, have been shown to have more or less bisexual properties, the corpus luteum hormone has long been considered specifically female in its action (Korenchevsky, 1937). With the advent of crude estrogen-free corpus luteum extracts (progestin, Allen and Meyer, 1933), pure crystalline hormone from extracts (Slotta, Ruschig, and Fels, 1934; Allen and Wintersteiner, 1934; Butenandt, Westphal, and Hohlweg, 1934), and synthetic progesterone (Fernholz, 1934; Butenandt and Schmidt, 1934; Butenandt and Westphal, 1934) it became more and more feasible to put to the test this assumption of sex specificity of the corpus luteum hormone. The experiments reported herewith were designed to determine whether definite effects follow the administration of progestin and progesterone in male rats.[3]

[1] This investigation has been aided by a grant from the Rockefeller Foundation to the University of Chicago.

[2] Presented before the American Society of Zoölogists at the Indianapolis meeting, December, 1937. Abstract in *Anatomical Record*, **70**, Supp. 1, pp. 45–46.

[3] It is a genuine pleasure to express my grateful appreciation to Professor Carl R. Moore for suggesting this problem and for his ever helpful co-operation and stimulating advice. To Professor W. C. Allee, my best thanks are due for use of essential equipment for hormone extractions, and to Professor Sewall Wright, my sincere gratitude for aid in the statistical analysis of the results. Grateful acknowledgment is hereby offered to the following for generous supplies of hormone preparations: Dr. Gregory Stragnell, of Schering Corporation, for Proluton and Oreton; Dr. J. F. Cartland, the Upjohn Company, for Progestin; and Dr. Oliver Kamm, Parke, Davis and Company, for Theelin.

Materials and Methods

We have used in these experiments: (1) sow corpus luteum extracts prepared in this laboratory ("Progestin I" and "Progestin II"); (2) sow corpus luteum extracts from the Upjohn Company ("Progestin III"); and (3) synthetic progesterone (Proluton, Schering). Progestin preparations had been subjected to treatment (Allen and Meyer, 1933) for removal of estrogens. Estrone (Theelin, Parke, Davis and Company) and testosterone (Oreton, Schering) were used for a comparison of their effects with those of corpus luteum hormone.

All corpus luteum hormone preparations were assayed on adult virgin female rabbits according to the techniques of Corner and Allen (1929) and Allen (1930).

Albino rats used in these experiments were of two kinds, namely, (1) an inbred strain derived from the regular colony, and (2) rats from the regular colony, used when inbred rats were not available. Female mice used as recipients of pituitary implants were from the small colony maintained in this laboratory for such purposes. Rabbits used for the assay of corpus luteum hormone were young mature virgin does purchased from a dealer and kept isolated in separate cages for a period of 1 month or more before treatment.

One hundred and ninety rats, 47 mice, and 21 rabbits were used in this investigation.

Methods standard in this laboratory for operation, injection, implantation of pituitaries, and autopsy were adopted. Histological methods were simple, involving paraffin imbedding and simple staining.

With the exception of assay work on rabbits, the principle of littermate controls has been used throughout. Analyses of organ weight data were carried out using Student's method.

Experimental Results

Progestin on normal young male rats

To determine the possible effects of corpus luteum hormone on normal rats a wide dose range of progestin was tried on four litters of various ages (see Table 1). Young

TABLE 1

Expt. No.	Dose Range (Rb. U. [Corner], Daily)	Progestin Sample Used	Injection Period (Days)		Age at Autopsy (Days)
			Age	Total	
I	1, 1/5, 1/20	I	38–51	14	52
II	1, 1/2	I	25–39	15	40
III	1, 1/2	I	17–31	15	32
IV	3	II	25–39	15	40

rats were used, for it is well known that young animals are more responsive to sex hormone treatments than are adults. In the first experiment a dose range of 0.05–1.0 rabbit unit (Rb. U.) (Corner) was tried, with the idea that some notion of threshold, and perhaps a quantitative difference in response, might be obtained should any effects of progestin be found. In the last experiment the largest dose which our limited supply would permit was given to one animal.

WEIGHT EFFECTS

Results of these experiments were, in the main, negative. Organ weights of treated animals differed from those of oil-injected littermate controls in but few cases. Even where some organ weight difference was observed, it could be considered only as suggestive because of the small number of animals used (one injectate at each dose-level). A brief outline of weight effects is detailed in Table 2.

TABLE 2

EFFECTS OF PROGESTIN ON ORGAN WEIGHTS ON THE BASIS OF ABSOLUTE WEIGHT AND PERCENTAGE OF BODY WEIGHT

Expt. No.	Percentage of Decrease (−) or Increase (+) in Organ Weights of Treated versus Control Animals											
	Body Weight	Testis	Preputial Glands	Ventral Prostate	Seminal Vesicles	Coagulating Glands	Perirurethral Tissue	Cowper's Glands	Adrenal Glands	Thymus Gland	Thyroid Gland	Pituitary Gland
I.....	o*	o	—†	o	o	o	—	o	o	—	o	o
II.....	o	o	—	o	o	o	—	o	o	o	o	o
III.....	o	−42, −46	—	+51, +61	+60, +70	+59, +69	—	o	o	−19, −24	o	o
IV.....	o	o	+50, +80	+99, +135	o	o	o	o	o	o	o	o

* Zero (o) indicates essentially no difference.
† Dash (—) indicates no weight taken.

A study of these data shows that in only one experiment (III), run from day 17 to day 32, was there any effect of injections on testis weight. This effect was a slowing of the growth-rate of the testis. At the same time there seemed to be a slowing of the growth-rate of the thymus and some stimulating effect on the growth of ventral prostate, seminal vesicles, and coagulating glands. In one other case (Expt. IV) the ventral prostate was distinctly heavier than that of the control. Preputial glands were heavier also. These weight differences were confined to organs which respond to androgens by similar changes. Therefore, they suggested a weak androgen-like effect of the injected material. None of the weight effects noted was very pronounced, and not enough animals were used to make the study of any response statistically significant.

EFFECTS ON SPERM MOTILITY

Sperm smears were taken at the time of autopsy of animals in Experiment I. In the animal which had received the highest dosage of progestin, spermatozoa were more numerous and more motile than in the animals receiving the lower dosages or in the oil-injected or untreated littermate controls. Animals in succeeding experiments were too young at autopsy to have spermatozoa in the epididymides or vasa deferentia.

EFFECTS ON GONADOTROPIC POTENCY OF PITUITARY

At the autopsy of animals in Experiments I and II the pituitaries were fixed for histological study. However, when the animals in Experiments III and IV came to autopsy, the pituitary gonadotropic potencies were tested by the implant method. In

Experiment III there appeared to be a definite depression in gonadotropic potency of pituitaries of both injected animals, as judged by ovarian and uterine weight differences in the implanted mice. In Experiment IV no such depression was apparent.

HISTOLOGICAL EFFECTS

Histological studies were made of certain organs from the experimental animals and their controls, to determine whether histological changes paralleled weight changes. A histological study of the testes was begun to determine what effect, if any, administration of progestin might have on sperm-head formation. Moore (1936) has shown that the time of appearance of sperm-heads in the testes of normal male rats of this colony is fixed at 33–35 days *post partum*. He found that this time of appearance was unaffected by injections of various gonadotropic preparations.

Experiments II, III, and IV were designed to test what effects administration of progestin might have on sperm-head appearance time. Two experiments (II and IV) were employed to test for any inhibitory action of the hormone on sperm-head formation within the dose range used. Injections were begun on day 25 and carried through day 39. Autopsy was on day 40. Oil-injected and normal littermate controls were run, and one normal control in each experiment was killed on day 33 to make sure that sperm-heads were not appearing extraordinarily early. It was decided that inhibition of sperm-head appearance would have to extend for about 5 days to be significant. Histological study showed that sperm-heads appeared by day 40 in all animals, including those treated with progestin. No sperm-heads were found in controls autopsied on day 33. Therefore, there was no apparent inhibitory effect from the dosages employed (0.5–3.0 Rb. U. daily) on the process of sperm-head formation.

Experiment III was planned to test for any accelerating action of progestin on sperm-head formation within the dose range employed. Injections ran from day 17 through day 31; autopsy was on day 32. Two dose-levels were used (0.5 and 1.0 Rb. U. daily). Oil-injected and normal littermate controls were run, and one normal control was allowed to live until day 36 to determine whether sperm-heads had formed by that time. Under these conditions there was no accelerating effect on sperm-head formation from administration of progestin. No sperm-heads were found in the testes of treated or control animals autopsied on day 32, whereas sperm-heads were found in the testes of the control autopsied on day 36. In the animal receiving 1 Rb. U. of progestin daily, not only was there no acceleration in the rate of sperm-head formation, but testis tubule diameters were definitely smaller than those of the normal littermates of the same age (ocular micrometer measurements). The testis weight was also far below normal (see Table 2).

The indications were, from these experiments, that progestin in the dosages employed had no effect on sperm-head appearance time but that testis weight and testis tubule diameter might be deleteriously affected.

Histological studies of other organs taken failed to reveal any striking differences correlated with progestin treatment. Organs studied included: (1) the sex accessories, such as ventral prostate, seminal vesicles, coagulating glands, periurethral tissue, Cowper's glands, and vas deferens; and (2) glands of internal secretion, such as adrenals, thymus, thyroids, and pituitary. Especial care was taken with the study of the pituitary, since Charipper (1934) had found what he called "pregnancy cells" in the pituitaries of normal and castrated male rats injected with 0.5 cc. of Lipolutin (Parke, Davis and

Company) for 15 days. According to Charipper, pregnancy cells are "large, ovoid, deeply eosin stained cells with homogeneous cytoplasm surrounding a usually eccentrically placed vesicular nucleus." Pituitaries of our experimental animals and their controls, and pituitaries of normal and pregnant female rats, were prepared according to Charipper's technique (Zenker fixation, Delafield's haematoxylin, and eosin). Eosinophilic cells were measured and studied for shape, for depth of stain, for homogeneity of cytoplasm, and for eccentricity of nucleus, without any special cell type having been found. Stein (1933, 1934) states that there are no special pregnancy cells in the rat. Other workers do not agree; nor do they agree on a definition of the term "pregnancy cell." Charipper (personal communication, 1937) now recognizes the error of the claims made in the 1934 paper.

EFFECTS OF CORPUS LUTEUM HORMONE ON CASTRATED YOUNG MALE RATS

It has been shown above that certain sex accessories of normal young males underwent a slight hypertrophy correlated with the administration of progestin. This and other findings suggested that the injected material might have a slight androgen-like effect. The normal male—even though immature—is useless as a test animal for androgens, because the animal's own gonads may be elaborating physiologically similar substances. Therefore, it was deemed advisable to cease all experiments on normals and to try injections of corpus luteum hormone into castrates to determine whether our preparations had any androgenic potency.

TREATMENTS

Littermates were castrated on the twenty-fifth day of life; and daily injections of corpus luteum hormone into some, of oil into others, were begun immediately. In all cases a normal littermate control was run. In most cases an uninjected castrated littermate control was run also. All treated animals and oil-injected controls were injected for 15 days, and all animals were autopsied on day 40. Progesterone was injected into four animals, in daily doses of 1, 2, 2, and 3 Rb. U. "Progestin II" was administered to one animal, 3 Rb. U. daily; and "Progestin III" was injected into one animal, also 3 Rb. U. daily. Six paired comparisons, therefore, were possible between treated castrates and their oil-injected castrated littermate controls.

WEIGHT EFFECTS

Organ weight data have been analyzed on the basis of such paired comparisons. Because body weight was affected, a new analysis was made to take this factor into account. That is to say, each organ weight was converted into percentage of body weight, and the paired comparisons were made anew. A summary of the two analyses is presented in Table 3, together with results of a determination by Student's method of the statistical significance of the results. Despite the fact that dose-level and character of preparation were not constant for all six injectates, the six paired comparisons were considered as a unit.

It will be noted that sex accessories were, in general, heavier than the corresponding organs of the castrated controls. This was particularly true of the ventral prostate, preputial glands, and periurethral tissue. Coagulating glands were consistently heavier in the treated animals, whereas seminal vesicle weights showed such consistency only when analyzed on the basis of percentage of body weight, and Cowper's glands showed it not at all. Adrenal glands showed some suggestion of being lighter in the treated

animals, and the thymus gland was consistently lighter. Thyroids were, on the average, slightly heavier in the treated animals, and pituitary weight followed body weight closely.

TABLE 3

STATISTICAL STUDY OF THE EFFECTS OF CORPUS LUTEUM HORMONE ON THE ORGAN WEIGHTS OF CASTRATED YOUNG RATS

Organ Weighed	On Basis of Absolute Weight			On Basis of Percentages of Body Weight		
	Mean Δ* (Per Cent)	Range of Δ (Per Cent)	*P*	Mean Δ (Per Cent)	Range of Δ (Per Cent)	*P*
Body weight.....	− 9	− 17 to − 3	.013			
Preputial glands...	+ 82	+ 22 to + 124	.008	+ 99	+ 42 to + 131	.003
Ventral prostate...	+684	+300 to +1,329	.005	+746	+380 to +1,383	.003
Periurethral tissue.	+ 73	+ 12 to + 183	.040	+ 90	+ 34 to + 228	.034
Coagulating glands	+ 43	+ 4 to + 82	.025	+ 57	+ 15 to + 109	.013
Seminal vesicles...	+ 19	− 2 to + 64	.600	+ 31	+ 5 to + 90	.065
Cowper's glands...	+ 27	− 9 to + 55	.053	+ 38	− 6 to + 65	.023
Adrenal glands....	− 18	− 26 to − 11	.001	− 9	− 24 to + 2	.065
Thymus gland.....	− 24	− 42 to − 5	.005	− 17	− 32 to − 2	.010
Thryoid glands....	+ 11	− 5 to + 38	.600	+ 23	− 2 to + 66	.069
Pituitary gland....	− 11	− 23 to − 2	.019	− 1	− 11 to + 12	.940

* Δ = Difference between injectate and control.

Since *P* represented the probability coefficient, and a *P* of .05 or less was taken as evidence of statistical significance, it may be concluded that there was a significant weight increase in the ventral prostate, preputial glands, periurethral tissue, and coagulating glands of treated castrates. The weight increase in Cowper's glands was probably significant, whereas the weight increases of the seminal vesicles approached significance only when analyzed on the basis of percentage of body weight. The thymus was significantly lighter in treated animals; the adrenals were probably significantly affected; and the body weight proved significantly lower in treated castrates. Thyroids and pituitary seemed to be less affected.

As an additional check on the validity of the results and to show that such results could not be the effect of random sampling, a series of twelve paired comparisons was made between castrated littermates receiving identical treatment (oil injections). The reference animal of each pair was chosen at the time of castration (day 25), in order to have a fixed basis of comparison and to avoid the possibility of later selection of animals in such a fashion as to make plus and minus weight differences balance. Autopsy was on day 40. Treatment of the data by statistical methods identical with those used above showed that there was a strikingly close agreement between littermates treated identically, and that in only one instance, and by only one method of analysis, did a probability coefficient fall below .05.[4] Even this result fits well into the array of random significance figures which one would expect on the basis of chance.

[4] Seminal vesicle, on the basis of absolute weight (*P* was .015). On the basis of percentage of body weight the *P* was .174. The range of the other probability coefficients was, on the basis of absolute weight, .260–.759, and on the basis of percentage of body weight, .201–.915.

Moore (1937) has called attention to the androgen measure available in littermate-controlled experiments, made possible by comparing the growth of end-organs in treated castrates with that of normal littermate controls. To determine what accessory sex organs were kept at or near the normal weight-level by corpus luteum hormone in the doses employed, the organ weight data were analyzed on the basis of paired comparison between treated castrates and their normal littermate controls. The following general conclusions were drawn: (1) preputial glands, ventral prostate, and periurethral tissue weights were maintained at or near the organ weight levels of the normal controls; and (2) coagulating glands, seminal vesicles, and Cowper's glands were definitely lighter than the corresponding organs of the normal controls.

COMPARISON OF WEIGHT EFFECTS OF CORPUS LUTEUM HORMONE AND OTHER SEX HORMONES

A comparison of organ weight responses was made between progesterone-treated castrates and testosterone-treated castrates. A graded series of testosterone was used in an endeavor to find a dose range giving effects comparable to those following progesterone treatment. Dilute solutions of testosterone in sesame oil were injected subcutaneously in daily doses of 0.03 to 0.10 mg. from castration on day 25 through day 39. Autopsy was on day 40. Comparison of the pooled data with data in Table 3 showed that testosterone, even in 0.10-mg. doses, did not cause a weight increase for preputial glands or ventral prostate equal to that produced by 1–3 Rb. U. of corpus luteum hormone. On the other hand, testosterone in 0.08-mg. doses caused about the same periurethral tissue increases as did corpus luteum hormone in the doses used; and as little as 0.03 mg. of testosterone daily caused more growth of coagulating glands, seminal vesicles, and Cowper's glands than did the doses of corpus luteum hormone used. Pituitary and thymus weights were slightly depressed by all doses of testosterone used, though the statistical significance of this finding was not tested. Adrenal and thyroid weights were not affected in either direction by such doses of testosterone at this stage.

The comparative effects of testosterone and progesterone treatment were analyzed in another way: comparison of the organ weights of testosterone-treated castrates with those of their normal littermate controls made possible deductions as to how much testosterone was necessary to keep the sex accessories at, or near, the normal weight-level. It was concluded that at this age, about 0.06 mg. of testosterone performed this function for most of the castrate sex accessories. A dose-level below 0.06 mg. failed to do this, and higher dose-levels gave heavier-than-normal accessory weights. All sex accessories except the ventral prostate responded to testosterone by comparable weight increases for any one dose-level. Ventral prostate weight was comparatively lower than that of any other accessory. Comparison of this action of testosterone with the action of corpus luteum hormone (see above) showed great differences in the stimulating potencies of the two hormones on different end-organs.

A better method of analyzing such differences in the two hormones, perhaps, is the method of prostate–seminal vesicle ratios used by Moore and Price (1937), Moore (1937), and Moore and Price (1938). The prostate–seminal vesicle ratio is obtained by dividing the total weight of the entire prostate complex (ventral prostate, coagulating glands, and periurethral tissue) by the weight of the seminal vesicles. It has been shown that the ratio decreases with age in normal rats, until it reaches 1/1 at sexual maturity, and that both androsterone and testosterone are able to produce any normal ratio in castrates, provided proper doses are used. Low doses produce high ratios, and vice versa.

Data for progesterone-treated and testosterone-treated animals and their controls were analyzed to determine the prostate-seminal vesicle ratio. Five castrates treated with 0.06 mg. of testosterone daily gave ratios averaging 6.5, or about the average for the normal controls (6.76). On the other hand, six castrates treated with corpus luteum hormone gave ratios averaging 18.3. It is to be remembered that each of the treated groups differed from the normals hardly at all in the weights of three accessories (preputial glands, ventral prostate, and periurethral tissue), but that the seminal vesicles of the progesterone-treated castrates were decidedly below normal in weight. This suggests that the corpus luteum hormone may adequately stimulate certain male end-organs without materially affecting others, in a fashion which is at variance with the action of testosterone.

A comparison was made of organ weight responses of progesterone-treated castrates and of estrone-treated castrates of similar age. A graded series of estrone-treated castrates was run, sometimes using littermates of the progesterone-treated animals, sometimes using different litters. Dilute estrone was injected in oil in graded doses (2–20 international units daily) from day 25 to day 39, with autopsy on day 40. In general, small increases, not correlated with dose-level, were found in organ weights of preputial gland, ventral prostate, coagulating gland, and periurethral tissue of treated castrates, as compared with similar weights of oil-injected castrated controls. Seminal vesicle weights showed increases correlated with dose-level; and weights of Cowper's glands, adrenals, thymus, thyroids, and pituitary showed no definite trend correlated with treatment.

In experiments with all three hormones, untreated castrated controls showed organ weights entirely comparable with those of oil-injected castrated controls. However, for the sake of uniformity, oil-injected castrated controls have been used for comparison with treated animals.

HISTOLOGICAL EFFECTS

Significant weight increases in certain accessory sex organs and weight decreases in the thymus of treated castrates led to the hypothesis that there might be an androgenic effect of corpus luteum hormone. Tissues taken at autopsy were divided into sex accessories and endocrine glands, and histological preparations were made. These two groups will be taken up in order.

SEX ACCESSORIES, OR ORGANS THAT MIGHT BE USED AS ANDROGEN INDICATORS

The ventral prostate.—The histology of the ventral prostate as an androgen indicator was worked out on the rat by Moore, Price, and Gallagher (1930); and a more careful investigation in young animals, both normal and castrated, was the work of Price (1936). She found that the normal prostate at 40 days is histologically similar to the adult gland, in that tubules are distended with secretion, gland cells are large and usually tall, and have "light areas" situated between the large basal round or oval nuclei and the free cell surfaces (see Fig. 1). These light areas are probably of significance in the physiology of secretion, for they are a constant feature of physiologically active cells, they disappear after castration, and they can be made to reappear with the administration of small doses of androgen. Consequently, they provide a sensitive test for the presence of androgens.

The prostates of animals castrated at 25 days and autopsied on day 40 showed striking differences from the normal picture (cf. Figs. 1 and 2). The tubular alveoli were smaller

and shorter. Tubular diameters were extremely reduced, as was also the amount of secretion within the tubule. Each gland cell was small, and practically cuboidal, with the nucleus near the center. Nuclei were smaller and more deeply stained. There was a relative (but probably not a true) increase in the intertubular connective tissue. Despite the fact that Price (1936) states that light areas persist in 30–40-day rats castrated during the first week of life, no light areas were found in animals castrated on day 25 and autopsied on day 40. Essentially the same thing was found by Greene, Burrill, and Ivy (1939).

In each comparison of normal control, castrated control, and corpus luteum hormone treated castrate, the ventral prostate of the treated castrate resembled, in all particulars, that of the normal control rat (cf. Figs. 1, 2, and 3). The tubules were distended with secretion; tubular diameters were fully as large as normal; and the gland cells were large, high columnar cells with basal nuclei. The nuclei were large and lightly stained. The proportion of tubular tissue and intertubular connective tissue was normal. Finally, light areas were found in the gland-cell cytoplasm between the nuclei and free cell borders.

Thus, it is apparent that the injected material, either corpus luteum extract or synthetic progesterone, acted as an androgen in building and maintaining the prostate in the normal secretory state.

Cowper's gland.—Heller (1930, 1932) worked out the histology of Cowper's gland as an androgen indicator. He says that by 40 days "the gland appears to have entered definitely upon its secretory activity." In adults, castration atrophy appears by 10 days, and is well advanced by 20 days, after castration.

In the normal 40-day-old rat Cowper's gland consisted of compound acini emptying into a central lumen. The entire gland was covered with a fibrous and muscular capsule. The central cavity and the gland duct were lined with a stratified epithelium. The acini were lined with tall columnar cells having small basally located, flattened nuclei, which were stained deeply with haematoxylin. The cytoplasm was very homogeneous and granular.

In rats castrated on day 25 and autopsied on day 40 Cowper's gland was much smaller than in the normal control. Considerable secretion still could be found in the central cavity, and there was a relatively greater amount of interacinous connective tissue and capsular material. Little secretory tissue was present. The few small acini were crowded between capsular connective tissue and the wall of the central cavity. Gland-cell height was much below that of the normal. Gland-cell nuclei were rounder and larger, less densely stained than in the normal. The condition was comparable to that of a much longer castration period in the adult (between 20 and 90 days).

In Cowper's glands of treated castrates the condition was intermediate between that described for the normal and for the castrated control. The secretory cells were higher, the acini were more numerous, and the interacinous connective tissue was relatively less prominent than in the oil-injected castrated control. Ocular-micrometer measurements showed that cell heights and acinar diameters were intermediate between those of the castrate and those of the normal control.

The vas deferens.—Vatna (1930) has worked out the histology and cytology of the vas deferens as an androgen indicator. Many changes due to castration were observed. He states that gross size is reduced, owing to a regression of the tunica muscularis. The amount of secretion within the lumen is diminished; and there is a reduction in epithelial cell height, a stratification of nuclei in the epithelial layer, obliteration of epithelial cell

walls, and a more or less complete loss of cilia-like structures from the epithelial surface. Where the "cilia" are not completely gone, they are short, matted, and disarranged.

In the castrate-control vas deferens at 40 days only some of the changes mentioned by Vatna were found. Obliteration of epithelial cell walls could not be seen in all, nor was there a difference in the amount of secretion within the lumen. Perhaps the more clearly defined changes from the normal in these animals were (1) reduction of the cilia-like processes, (2) reduction in gross size, and (3) apparent stratification of epithelial nuclei. Nuclei in the epithelial cells of castrates tended to be more basal than is normal.

Corpus luteum hormone treated castrates showed a histology and cytology of the vas deferens entirely comparable to the normal control. Lumina were large, and the epithelial cells retained their size and their long cilia-like processes. Gross cross-sectional size of the vas deferens was essentially that of the normal control. Nuclei of the epithelial cells were usually located about two-thirds of the distance from the free border of the cell to its base, as was the case in the normal controls.

Seminal vesicle.—The seminal vesicle cytology test has been suggested as a male hormone indicator by several workers. As worked out by Moore, Hughes, and Gallagher (1930), a positive test depends on the presence of secretion granules in sections of the seminal vesicle stained with Ehrlich's haematoyxlin. Castration causes a disappearance of secretion granules from the adult seminal vesicle within 48 hours.

At 40 days the normal controls of the experimental series were at the beginning of their adult secretory state. Epithelial cells were not so high as in the adult, and secretion granules with halos were not so prominent or so frequent as in the adult. Secretion granules were, however, undoubtedly present by 40 days (Price, 1936, says they appear by day 36), and secretion could be seen in the lumen of the gland.

The condition of the castrate-control seminal vesicle at 40 days was distinctly different from that of the normal control. The cavity of the gland was much smaller and was not filled with a stainable secretion. The blind out-pouchings of the central cavity were not extensive. Secretory epithelium showed a severe castration atrophy. The cells were low, scarcely higher than the diameter of the nucleus; and no secretion granules were present.

Treated castrates showed essentially the same seminal vesicle histology and cytology as did the castrated controls. Epithelial cells were low, and there was practically no cytoplasm distal to the nucleus. In one case only was stainable secretion found in such a seminal vesicle. Search revealed a few secretion granules in a consistently higher epithelium. This was in RE175, an animal which received .3 Rb. U. of "Progestin III" daily, in dilute solution. Seminal vesicle weight increase was also the most pronounced (+90 per cent) of any animal in the series.

Middle lobe of the prostate (*periurethral tissue*).—Korenchevsky and Dennison (1935) have divided this prostatic tissue into three general subdivisions: lateral, dorsal, and central lobes. Moore (1939) mentions lateral and dorsal subdivisions only. Histological study was confined to the lateral subdivision. Acinar structure somewhat different from that of the ventral prostate was found. Cell heights of the normal and castrate gland did not differ appreciably, but acinar diameters decreased following castration. Similarly, effects of corpus luteum hormone were not on cell height, but rather on acinar diameters. Acinar diameters in the lateral subdivision of the prostate of treated animals averaged well above those of both the castrate and the normal control.

The coagulating gland.—Moore, Price, and Gallagher (1930) found that in the adult

the coagulating gland responds to sex hormone states and is an additional, though less satisfactory, androgen indicator. Response to androgens is merely one of epithelial cell size and acinar size.

A detailed comparison between normal and castrated controls of the experimental series, autopsied on day 40, failed to reveal any histological basis in the coagulating gland for distinguishing between the two catagories of animals. Cell height measurements and acinar diameter measurements were not essentially different in the normal, castrated control or in castrated treated animals.

The preputial glands.—Preputial glands of adult rats respond to castration by certain well-defined histological changes, according to Korenchevsky and Dennison (1935). These are reductions in the number and size of the cells and alveoli. Cells become atrophic and more irregular. Vacuolization of the cytoplasm is coarser. Pyknotic nuclei are more numerous, and nuclei are packed closer together. Canals and ducts are smaller, are less ramified, and contain less secretion.

None of these changes were found in castrated controls of the experimental series, whether because of early age at castration and autopsy, because of short castration time, or because of some other factors we do not know. Neither was any histological change noted in the preputial glands of treated castrates.

ENDOCRINE GLANDS

Histological preparations of the adrenal gland, thymus, and thyroid were studied; but no striking changes attributable to either castration or corpus luteum hormone treatment were evident. Mammary gland and pituitary gland preparations have not been made.

A summary of the histological effects of corpus luteum hormone on castrates is presented in Table 4.

TABLE 4

Organ	Effects of Castration from Day 25 to Day 40	Effects of Hormone (1–3 Rb. U. Daily) into Castrates from Day 25 to Day 40
Ventral prostate	Castration atrophy	Prevention of castration atrophy; light areas present
Vas deferens	Castration atrophy	Prevention of castration atrophy
Cowper's glands	Castration atrophy	Amelioration of castration atrophy
Seminal vesicles	Castration atrophy	Slight repair in one case only
Middle prostate	Decrease in tubular diameters	Tubular diameters larger than in castrate or normal controls
Coagulating glands	No effects	No effects
Preputial glands	No effects	No effects
Adrenal glands	No effects	No effects
Thymus gland	No effects	No effects
Thyroid glands	No effects	No effects

EFFECTS OF IMPLANTS OF PITUITARIES INTO MICE

Three experimental series of animals had the pituitary gonadotropic potency assayed on 21-day female mice. No depression or augmentation of gonadotropic potency was observed which could be correlated with corpus luteum hormone treatment.

Discussion

It has been seen in the preceding section that certain tests on male rats indicated a weak androgenic activity of corpus luteum extracts and of synthetic progesterone. A critical search of the literature published prior to the presentation of the preliminary report of this work failed to reveal claims of androgenic effects of corpus luteum hormone in males. In fact, most of the workers dealing with the subject of corpus luteum hormone effects in males have been unable to demonstrate any outstanding effects of hormone administration. Greene, Burrill, and Ivy (1939) have been able to confirm and to extend some of the conclusions presented in the preliminary report of this work.

All articles dealing with possible effects of corpus luteum hormone on male individuals may be classified into one of three general groups: (1) those claiming no effects, (2) those claiming effects which have been brought into question by later work, and (3) those claiming effects which have not been questioned. Of the first group, the work of Fels (1935), of Albrieux, Buño, Engel, and Morató-Manaro (1936), of Fels (1936, 1937), and of Korenchevsky (1937) may be mentioned. As examples of the second group, the papers of Charipper (1934) and of Clauberg and Breipohl (1935) are cases in point. These authors claimed that corpus luteum extracts correct castration cells in the pituitaries of castrated rats. Fels (1935) and Hohlweg (1935), using larger doses of crystalline progesterone, failed to confirm this observation. They suggested that the results of the original workers may have been due to estrogenic impurities in their preparations. Charipper (1934) claimed also that "pregnancy cells" can be formed in the pituitaries of either normal or castrated male rats by administration of corpus luteum extracts. The work of Stein (1933, 1934) and the admission of Charipper (personal communication, 1937), already noted, dismiss this assertion. Examples of effects which have been reported but not questioned are the following: (1) Gardner and Hill (1936) have been able to produce mammary gland growth in normal and castrated male mice by corpus luteum treatment; (2) Zuckerman and Parkes (1936) found that the administration of progesterone to male monkeys causes no change in the prostate or other sex accessories but does tend to correct the pathology of the lining of the uterus masculinus which has resulted from injected estrone; (3) Burrows (1936) claimed that progesterone protects normal male mice, but not castrates, from the harmful effects of applied estrone; and (4) Nelson (1936, 1937) found that corpus luteum hormone is as effective as androgens in maintaining spermatogenesis in hypophysectomized rats but that no androgenic effects are evinced either by the injected material directly or by indirect stimulation to production of testicular internal secretion.

Whereas there were no previous claims in the literature that corpus luteum extracts or progesterone in males act as androgens, there were certain indications that such might be the case and certain findings which can be explained better, perhaps, by such an assumption. Steinach and Kun, as early as 1931, found that in the female guinea pig transformation of the clitoris into a penis-like organ results from any one of three kinds of treatment, namely, (1) X-irradiation of the ovaries, followed by luteinization; (2) administration of gonadotropic extract, followed by luteinization; or (3) administration of luteal extracts (into spayed or normal females). In all cases the level of corpus luteum hormone in the blood would be raised. Hill (1937*a*, 1937*b*, and 1937*c*) found that ovarian grafts in the ears of castrated male mice luteinize and that, where such grafts are present, the castrate accessories are repaired. Price (personal communication, 1937)

obtained "functional appearing" ventral prostate grafts when the hosts were either normal virgin female rats or female rats kept pregnant. Grafts are larger, on the average, in the latter hosts. Parkes (1937), using a very sensitive comb-growth test and extraction methods similar to our own for extracting corpus luteum hormone, found that certain ovarian extracts are slightly but definitely androgenic. Finally, as very good evidence, Steinach and Kun (1931) administered luteal extracts to young castrated male rats and produced normal-looking penis development. They thought that the corpus luteum produces not one hormone but two, namely, (1) a hormone for the production of a progravid uterine endometrium, and (2) a hormone identical with testis hormone. All these evidences fit into the general picture and are unified by the results of the present experiments.

A study of comparative effects of progesterone and testosterone suggests that the two may differ somewhat in their physiological expression, although both may act as androgens. A great deal more evidence is needed to corroborate this suggestion, but it does present some interesting implications. That androgens may differ among themselves in relative potency, and that a single androgen may show threshold differences with regard to different end-organs, is well known. It has also been strongly suggested (see Deanesly and Parkes, 1936; Korenchevsky, Dennison, and Brovsin, 1936; and Tschopp, 1936; and their citations) that androgens may differ among themselves as to their relative threshold differences—that is, a certain hormone may exhibit a "preferential" action on an end-organ out of all proportion to its effects on other end-organs, as compared with other androgens. Moore (Moore and Price, 1937; Moore, 1937; and Moore and Price, 1938) has brought this idea into question with his work on prostate–seminal vesicle ratios, and the problem is still unsettled. It is very certain that progesterone exhibits threshold differences for different end-organs in the castrated young male rat. Whether such differences are comparable with those exhibited by testosterone or are actually preferential for prostate tissue over and above those of testosterone, as they seem to be, remains for further work to decide.

There remains the question, still unanswered, as to whether progesterone acts as an androgen per se or is changed in the body by relatively simple chemical reorganization into an androgen. It is true that progesterone is more closely similar chemically to certain androgens, i.e., testosterone and androstenedione, than are these hormones to other androgens. The simplest change of progesterone to any of the known androgens would probably be to testosterone. However, since the physiological expression of the two (testosterone and progesterone) seems to be dissimilar, it is unlikely that this change takes place. It seems quite as possible that the progesterone acts per se as a weak androgen.

It is not unlikely that still other weight responses and histological changes may be found to result from progesterone treatment when more extensive work is done, using more animals and larger doses of the hormone. When considered on the basis of percentage of body weight, the adrenals and thyroids of treated castrates showed weight decreases approaching a statistically significant value. Also, it is possible that with larger doses the seminal vesicles may be affected in the same manner as are the other sex accessories.

That sex accessories, which would be expected to act in similar fashion under sex hormone stimulation, showed, in general, weight increases, whereas other organs showed no change in weight and still others showed weight decreases, adds to the dependence to

be put in the results. Confirmation is to be had in histological changes correlated with treatment. All positive effects indicate a weak androgen-like activity of progesterone.

That the effects seen are due to progesterone alone, and not to any other substance, seems indicated by the following facts: (1) controls injected with plain oil showed none of the effects; (2) all extracts used were treated to remove estrogens; (3) most of the work on castrates was done using synthetic crystalline progesterone, both the extracts and pure progesterone giving the same results; and (4) the comparison of progesterone effects with effects of estrone showed a decided qualitative difference in the two hormones.

Korenchevsky (1937) has advanced the idea that sex hormones can be classified into one of three general categories, namely, (1) monosexual, (2) partly bisexual, and (3) true bisexual hormones. Most hormones fall into the bisexual groups. In fact, progesterone alone was placed in the monosexual category as a purely female hormone. This idea has been controverted by the present experiments, and it may now be said that we know of no purely monosexual hormone.

Summary

1. Results of administration of corpus luteum extracts to normal young male rats suggested a weak androgenic action of the preparations.

2. Within the dose ranges employed ($\frac{1}{2}$–3 Rb. U. [Corner] daily for 15 days) there was no slowing or acceleration of the rate of sperm-head formation due to the injected corpus luteum extracts.

3. Progesterone or corpus luteum extracts injected (1–3 Rb. U. [Corner] daily for 15 days) into castrated young male rats caused (1) a significant weight increase for preputial glands, ventral prostate, periurethral tissue, coagulating glands, and possibly Cowper's glands; (2) a significant weight decrease for body weight and thymus gland; and (3) no decided weight change for seminal vesicles, adrenals, thyroids, and pituitary.

4. Difference in grade of weight response of the various sex accessories to progesterone may be attributed to a threshold phenomenon.

5. Comparison of weight effects of progesterone and testosterone suggests a difference between the two with respect to relative thresholds for prostatic tissue and the seminal vesicle.

6. Castrated sex accessories which responded histologically to progesterone and to corpus luteum extracts by amelioration of castration atrophy were, in order of decreasing amelioration: ventral prostate, vas deferens, lateral lobe of middle prostate, Cowper's glands, and seminal vesicles. Accessories which responded histologically neither to castration nor to injections were the coagulating glands and preputial glands. Adrenals, thymus, and thyroids showed no histological changes to castration or to corpus luteum hormone injections under the experimental conditions.

7. No difference in pituitary gonadotropic potency was observed between injected castrates and their controls.

8. All positive effects of progesterone and corpus luteum extracts in young male rats, castrated or normal, pointed to an androgenic potency of these preparations.

9. Since results of progesterone and corpus luteum extracts on castrates were entirely similar, and since injections of estrone gave very dissimilar results it may safely be concluded that all results were due to the administration of the corpus luteum hormone and not to any other hormone or impurity.

10. Prior to these experiments progesterone was the only sex hormone for which bisexual effects had not been demonstrated. Now it has been shown to have effects on accessory sex organs in the male.

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PLATE I

FIG. 1

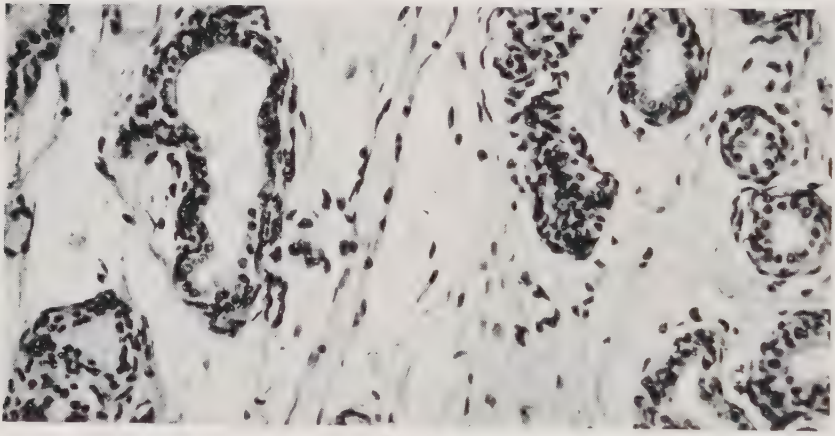

FIG. 2

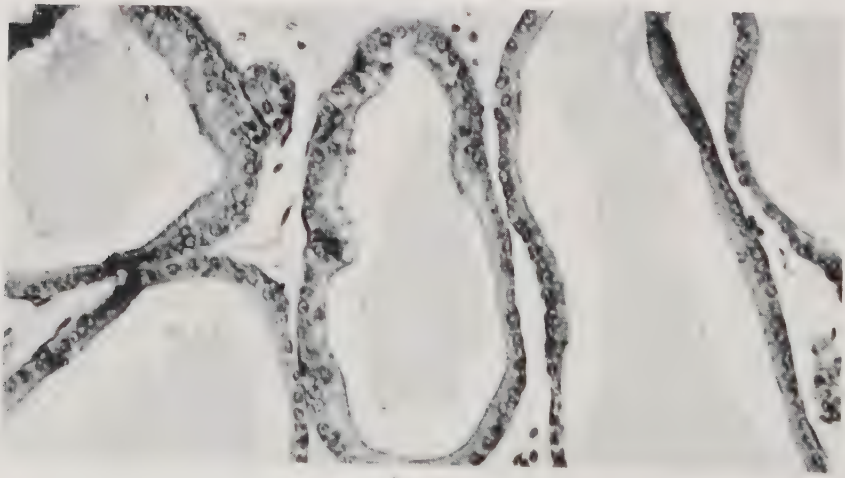

FIG. 3

PLATE I

FIG. 1.—Section of a normal ventral prostate at 40 days. ′×260.

FIG. 2.—Section of the ventral prostate of a littermate, castrated from day 25 to day 40. ×260.

FIG. 3.—Section of the ventral prostate of a littermate castrated, and injected with 2 mg. of progesterone daily, from day 25 to day 40. ×260.